REMARKS

The present application is a Rule 60 continuation application which is entitled, under 35 U.S.C. § 112 and § 120, to the benefit of the earlier filing dates of application Serial No. 07/787,390 filed November 4, 1991 ("'390 parent application"), and application Serial No. 432,069 filed November 6, 1989 ("'069 grandparent application").[1/] The specification has been amended to identify and update the status of the '390 parent and '069 grandparent applications under 35 U.S.C. § 120, and to correct errors as requested by the Examiner.

1. **The Claimed Invention**

The present invention involves the use of targeted homologous recombination to accomplish gene activation and/or to enhance gene expression in mammalian host cells. To this end, targeting vectors are designed and used to integrate a regulatory element and/or an amplifiable gene into a mammalian host cell genome in a region located within or proximal to a target gene of interest. Integration of the regulatory

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1/ The '390 parent application is a continuation-in-part application filed as the national stage of International application PCT/US90/06436 filed November 6, 1990. In the Request for filing the present Rule 60 continuation application, the specification was amended to contain a reference to the '390 parent application (Request, Box 7), and claimed priority under 35 U.S.C. §119 (Request, Box 9a). The claim to priority under 35 U.S.C. § 119 was unnecessary since the '390 parent application is a U.S. application. For reasons detailed herein, the present application is entitled to the earlier filing dates of the '390 parent and '069 grandparent applications under 35 U.S.C. §120.

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element in operative association with the target gene results in gene activation and/or modified or enhanced expression, i.e., the integrated regulatory element will drive expression of the target gene in the host cell. Integration of an amplifiable gene proximal to the target gene allows for amplification of the target gene, which increases gene expression.

The mammalian host cell so modified by targeted homologous recombination expresses the activated and/or amplified gene product. However, in certain situations, e.g., where the mammalian host cell is a primary cell that does not grow readily in culture, the activated gene can be transferred to a secondary expression host cell that is more efficient for large scale production.

The claims have been amended to more particularly point out and distinctly claim the subject matter of the invention. In particular, Claims 26, 48, 62, 67 and 69 and claims dependent therefrom, and new Claims 72, 76, 83, 93, 97 and 101 have been added to refer to the regulatory element as an "integrated" regulatory element that controls expression of the endogenous target gene, as disclosed in the instant specification and the '390 parent application at p. 7, lines 1-35, and in the '069 grandparent application at p. 6, line 25 to p. 7., line 20. New dependent Claims 73-74, 77-78, 84-85, 89-90, 95-96 and 103-104 further specify that the regulatory element is a promoter, promoter/enhancer, or enhancer as disclosed in the applications above, or the CMV

promoter/enhancer as disclosed in the instant specification on p. 15, lines 23-29.

Claims 27, 32, 49, 63 and claims dependent therefrom, and new Claims 75, 82, 91, 92, 98 and 100 have been added to refer to the amplifiable gene as integrated into the host cell genome within or proximal to the endogenous target gene so that the endogenous gene is also amplifiable, and to clarify that the amplifiable gene can be engineered into the host cell genome with or without a regulatory element to control expression of the target gene as described in the instant specification and '390 parent application at p. 4, lines 5-29 and at p. 6, lines 6-38; and in the '069 grandparent application at p. 3, line 31 to p. 5, line 17, and at p. 5, line 27 to p. 6, line 24.

New Claims 79, 80, 81, 86, 87 and 88 have been added to include the use of selectable markers; e.g., neo gene/G418, herpes virus tk gene/HAT medium, gpt gene/mycophenolic acid; and negative selectable markers e.g., herpes virus tk gene/gangcyclovir or acyclovir as disclosed in the instant specification and '390 parent application at p. 9, lines 3-9 and 25-34; and p. 10, lines 10-22 and in the '069 grandparent application at p. 8, lines 16-30, p. 9, lines 11-20; and p. 9, line 32 to p. 10, line 7. Claims 37 and 51 have also been amended to indicate that the host cell is a "primary" host cell as described in the instant specification at page 4, line 30 to page 5, line 10.

Claims 33, 37, 38, 43, 44, 46, 50, 52, 57, 65 and 70 have been amended to correct dependencies. Claims 49, 62, and 67 have been amended to correct the lettering of subparts to the claims. Claims 32, 62 and 63 have been rewritten in independent form. New Claims 92-104 have been added to specify recovery of the recombinant protein from the mammalian host cells of Claims 27 and 32, and the secondary expression host cells of Claim 44 as described in the specification at p. 12, lines 7-16. Claims 1 and 45 have been cancelled, without prejudice, to eliminate redundancies. Claim 71, covering recombinant proteins has been cancelled, without prejudice.

The foregoing amendments are fully supported by the specification and claims as originally filed, and by the specification and claims of the '390 parent and '069 grandparent applications. No new matter is introduced by the amendments.

2. The Provisional Obviousness-Type Double-Patenting Rejection Should Be Withdrawn

The claims are provisionally rejected under the judicially created doctrine of obviousness-type double-patenting in view of Claims 1 and 20-56 of Applicant's co-pending application Serial no. 08/102,567 ("'567 application").

This rejection, albeit provisional, should be withdrawn because the inventions claimed herein and in

Applicant's co-pending '567 application are not obvious variants of one another.

The presently claimed invention involves the use of targeted homologous recombination to accomplish gene activation and/or amplification in a mammalian host cell; i.e., a regulatory element and/or an amplifiable gene is integrated into the host cell genome within or proximal to an endogenous target gene via targeted homologous recombination. The resulting activated, modified and/or amplified gene is expressed by the mammalian host cell, which may or may not be a continuous cell line. Where the mammalian host cell is a primary cell that does not grow readily in culture, the activated, modified and/or amplified gene can be transferred to a secondary expression host cell, e.g., a continuous cell line which may have better growth characteristics for large scale culturing purposes.

In contrast, the invention described and claimed in Applicant's co-pending '567 application involves the use of targeted homologous recombination to insert a regulatory element and/or amplifiable gene into or proximal to a target gene contained in a yeast artificial chromosome (YAC) in yeast host cells. The use of YACs engineered to contain the target gene allows one to take advantage of the high efficiency with which yeast host cells perform homologous recombination. However, unlike the present invention, the target gene itself is exogenous to the yeast host cell which does not express the target gene. Expression of the target gene is achieved when

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the recombined "activated" or modified target gene is transferred to a host which responds to the regulatory element, and/or when selection pressure is applied to the ultimate host cell used to amplify the amplifiable gene and the proximal target gene.

The use of the YAC system described in Applicant's co-pending '567 application does not suggest that homologous recombination could be used to successfully activate, modify and/or amplify endogenous genes in mammalian host cells where homologous recombination was viewed as an inefficient process. By the same token, the use of targeted homologous recombination to achieve gene expression by activating, modifying and/or amplifying a target gene endogenous to a mammalian host cell described and claimed in the instant application does not suggest the use of YACs to increase the efficiency of homologous recombination for "activating" or modifying foreign genes contained in the YAC as described and claimed in Applicant's copending '567 application. These fundamental differences between the two inventions are <u>not</u> merely obvious variations of each other. Therefore, the provisional obviousness-type double patenting rejection should be withdrawn.

3. The Amended Claims are Definite Under <u>35 U.S.C. § 112, Second Paragraph</u>

Claims 1, 37, 48-63, and 67-68 are rejected under 35 U.S.C. § 112, second paragraph as indefinite.

This rejection is obviated and/or overcome by the amended claims. In particular, Claim 1 has been cancelled, without prejudice; Claims 37, 48, 49, 51, 62, 63, 67 and 68 are amended to replace the phrases which the Examiner finds vague with language that better defines the location of the homologous region with respect to the target gene; and the lettering of the subparts of Claims 49, 62 and 63 have been corrected.

In view of the foregoing amendments, the rejections under 35 U.S.C. § 112, second paragraph, should be withdrawn.

4. The Claims are Entitled to the Benefit of the November 6, 1989 Filing Date of the '069 Grandparent Application Under 35 U.S.C. §§ 112 and 120

Claims 26-71 are rejected and the specification objected to under 35 U.S.C. § 112, first paragraph. The Examiner contends that the specification fails to disclose homologous recombination using an exogenous regulatory element, and has not accorded the claims the benefit of the priority date, but rather, the filing date of the instant application. For reasons detailed below, this rejection is in error and should be withdrawn, and the claims should be accorded the benefit of the November 6, 1989 filing date of the '069 grandparent application.

The specification of the instant application, which is identical to the specification of the '390 parent application originally filed on November 4, 1991, clearly teaches the use of targeted homologous recombination to

engineer an exogenous regulatory element into a gene of interest contained in the genome of a mammalian host cell. Indeed, the specification supports the use of targeted homologous recombination to engineer any regulatory element, exogenous or otherwise, into a gene of interest contained in the genome of a mammalian host cell. The Applicants have amended the claims to more precisely reflect the scope supported by the specification.

The Examiner's attention is invited, for example, to the Summary of the Invention at page 3, lines 12-17:

> "Expression of mammalian proteins of interest is achieved by employing homologous recombination for integration of an amplifiable gene and other regulatory sequences in proximity to a gene of interest without interruption of the production of a proper transcript." (emphasis supplied).

The specification explains that targeted homologous recombination is accomplished using vectors that include DNA sequences homologous to DNA flanking the transcribed region of the target gene, and specifically states that regulatory elements different than wild-type, i.e., exogenous regulatory elements, may be used; e.g., that exogenous enhancer elements may be introduced, or that the wild-type regulatory element may be mutated or modified. For instance, the Examiner's attention is invited to page 7, lines 3-35:

> For example, one may wish to change the transcriptional initiation region for the target gene, so that a portion of the homologous region might comprise nucleotides different from the wild-type 5' region of the target gene. Alternatively, one could provide for insertion of a transcriptional initiation region different from the wild-type region

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between the wild-type initiation region and the structural gene.

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Therefore, by homologous recombination, one can provide for maintaining the integrity of the target gene, so as to express the wild-type protein under the transcriptional regulation of the wild-type promoter or one may provide for a change in transcriptional regulation, processing or sequence of the target gene. In some instances, one may wish to introduce an enhancer in relation to the transcriptional initiation region . . . (emphasis supplied).

The foregoing teachings of the instant application relating to the use of exogenous or modified regulatory elements are identically disclosed in the '390 parent application (at p. 3, lines 12-17 and p. 7, lines 3-35), and in the '069 grandparent application (at p. 3, lines 16-21; and p. 6, line 27 to p. 7, line 21).

In both the '390 parent application and the instant specification, the use of an exogenous regulatory element as described above is illustrated in the working examples, where the CMV promoter/enhancer was introduced into the erythropoietin (EPO) gene by targeted homologous recombination (specification, p. 15, line 1 to p. 19, line 8; and Figs. 3-4).

The pending claims have been amended to clarify the invention with respect to the regulatory elements that can be used to engineer host cells. In particular, Claims 28-44, 46-47 and new Claims 72-74, which cover host cells containing the integrated regulatory element, specify that the element is one that is different from the wild-type element normally

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associated with the endogenous gene; e.g., a mutated or exogenous promoter or enhancer, such as the CMV promoter/ enhancer. Claims 48-61 and new Claims 75-78, covering methods for engineering the host cells containing the homologously recombined regulatory element refer to the element as an "integrated" element -- i.e., any regulatory element, whether endogenous or exogenous to the host cell, native or heterologous to the endogenous gene, can be employed in the method of the invention. The same amendments have been made to Claims 69-70 and new Claims 92-96 covering the use of such host cells to produce the recombinant protein encoded by the modified endogenous gene.

In view of the foregoing, the pending claims are fully supported within the meaning of 35 U.S.C. § 112, first paragraph, by the respective disclosures of the instant specification, the '390 parent application, and the '069 grandparent application. Therefore, under 35 U.S.C. § 120, the claims are entitled to the benefit of the earliest priority date, i.e., the November 6, 1989 filing date of the '069 grandparent application.

5. The Pending Claims are Neither Anticipated Nor Made Obvious by the Art

Claims 26, 27, 32, 43 and 69-71 are rejected under 35 U.S.C. § 102(b) as anticipated by WO91/09955 ("Chappel PCT application"). The remaining claims are rejected under 35 U.S.C. § 103 as obvious over the Chappel PCT application

combined with a variety of secondary references. These rejections are in error, and should be withdrawn.

The Chappel PCT application was published on July 11, 1991, and therefore, is not prior art to the instantly claimed invention which is entitled to the benefit of the November 6, 1989 filing date of the '069 grandparent application.

In view of the foregoing, all rejections under 35 U.S.C. § 102(b) and § 103 in view of the Chappel PCT application, whether considered individually or in combination with the secondary references, should be withdrawn.

6. An Interference Should Be Declared between the Present Application and U.S. Patent No. 5,272,071

The Applicant would like to bring to the Examiner's attention the U.S. counterpart of the Chappel PCT application: U.S. Patent No. 5,272,071 issued on December 21, 1993 to Chappel ("'071 Chappel patent"). The '071 Chappel patent issued from a series of applications, the earliest of which was filed December 22, 1989.[2/] Even assuming, *arguendo*, that the '071 Chappel patent were entitled to the benefit of the December 22, 1989 filing date under 35 U.S.C. § 112, the '071

[2/] The '071 Chappel patent issued from continuation-in-part U.S. application serial no. 893,447 filed May 28, 1992 as the national stage of International Application PCT No./USPO/07642 (WO91/09955) filed December 21, 1990 claiming priority benefits to U.S. patent application serial no. 454,783, filed December 22, 1989, abandoned. The §102(e) date indicated on the face of the '071 Chappel patent is May 28, 1992.

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Chappel patent is not prior art under 35 U.S.C. § 102 or § 103, because the November 6, 1989 effective date of the pending claims antedates the earliest effective date to which the '071 Chappel patent could possibly be entitled.

As set forth in detail in the accompanying Request for Interference, the '071 Chappel patent purports to claim the same invention as is covered by the pending claims. Therefore, an interference should be declared to determine who is the first inventor of the conflicting subject matter.

7. **Miscellaneous**

The Applicant would like to bring to the Examiner's attention International Application Number PCT/FR90/00185 (International Publication No. WO90/11354) by Le Mouellic et al. of the Institut Pasteur ("Le Mouellic PCT Application"), cited in the accompanying Supplemental Information Disclosure Statement.

The Le Mouellic PCT Application was published on October 4, 1990 and therefore, is not prior art to the instantly claimed invention which is entitled to the benefit of the November 6, 1989 filing date of the '069 grandparent application. However, the status of any corresponding United States application, if any exists, is unknown.

For the Examiner's convenience, a copy of the Le Mouellic PCT application, the French parent application and the English translations accompany the Supplemental Information Disclosure Statement submitted herewith.

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CONCLUSION

The Applicant requests entry and consideration of the foregoing amendments and remarks into the file of the above-identified application. For the foregoing reasons, all rejections of the claims should be withdrawn and the claims found allowable. For reasons detailed in the accompanying Request for Interference, an interference should be declared with the Chappel '071 patent.

Respectfully submitted,

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